Domestic Animal Endocrinology 45 (2013) 163–169

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Heat-tolerant versus heat-sensitive *Bos taurus* cattle: influence of air temperature and breed on the acute phase response to a provocative immune challenge

J.A. Carroll [a,*], N.C. Burdick Sanchez [a], R. Chaffin [b], C.C. Chase Jr [c,†], S.W. Coleman [c,‡], D.E. Spiers [b]

[a] *Livestock Issues Research Unit, USDA Agricultural Research Service, Lubbock, TX 79403, USA*
[b] *Division of Animal Sciences, University of Missouri, Columbia, MO 65211, USA*
[c] *SubTropical Agricultural Research Station, USDA Agricultural Research Service, Brooksville, FL 34601, USA*

ARTICLE INFO

Article history:
Received 25 April 2013
Received in revised form 12 August 2013
Accepted 12 August 2013

Keywords:
Angus
Romosinuano
Heat stress
Immune
Lipopolysaccharide

ABSTRACT

The difference in the acute phase response of a heat-tolerant and a heat-sensitive *Bos taurus* breed to a lipopolysaccharide (LPS) challenge when housed at different air temperatures (T_a) was studied. Angus (ANG; heat-sensitive; n = 11; 306 ± 26 kg BW) and Romosinuano (RO; heat-tolerant; n = 10; 313 ± 32 kg BW) heifers were transported from the USDA Agricultural Research Service SubTropical Agricultural Research Station in Florida to the Brody Environmental Chambers at the University of Missouri, Columbia. Heifers were housed in stanchions in 4 temperature-controlled environmental chambers. Initially, T_a in the 4 chambers was cycling at thermoneutrality (TN; 18.5°C–23.5°C) for a 1-wk adjustment period, followed by an increase in 2 of the 4 chambers to cycling heat stress (HS; 24°C–38°C) for 2 wk. On day 19, heifers were fitted with jugular catheters and rectal temperature (RT) recording devices. On day 20, heifers were challenged with LPS (0.5 μg/kg BW; 0 h), sickness behavior scores (SBSs) were recorded, and blood samples were collected at 0.5-h intervals from −2 to 8 h and again at 24 h relative to LPS challenge at 0 h. Serum was isolated and stored at −80°C until analyzed for cortisol and cytokine concentrations. A breed by T_a interaction ($P < 0.001$) was observed for RT such that the post-LPS average RT in RO heifers housed at TN was lower than the RT of all other treatment groups ($P < 0.001$), whereas ANG heifers housed at HS had greater post-LPS average RT than all other treatment groups ($P < 0.001$). In response to LPS, HS increased SBS after LPS in RO heifers compared to RO heifers housed at TN ($P < 0.001$), whereas HS decreased SBS after LPS in ANG heifers compared to ANG heifers housed at TN ($P = 0.014$). The cortisol response to LPS was greater in TN than in HS heifers ($P < 0.01$) and was also greater in RO than in ANG heifers ($P = 0.03$). A breed by T_a interaction ($P < 0.01$) was observed for tumor necrosis factor-α (TNF-α) concentration such that HS increased post-LPS serum concentrations of TNF-α in ANG heifers compared to ANG heifers housed at TN ($P = 0.041$),

Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

The U.S. Department of Agriculture (USDA) prohibits discrimination in all its programs and activities on the basis of race, color, national origin, age, disability, and, where applicable, sex, marital status, familial status, parental status, religion, sexual orientation, genetic information, political beliefs, reprisal, or because all or part of an individual's income is derived from any public assistance program. (Not all prohibited bases apply to all programs.) Persons with disabilities who require alternative means for communication of program information (Braille, large print, audiotape, etc) should contact USDA's TARGET Center at (202) 720-2600 (voice and TDD). To file a complaint of discrimination, write to USDA, Director, Office of Civil Rights, 1400 Independence Avenue, S.W., Washington, D.C. 20250-9410, or call (800) 795-3272 (voice) or (202) 720-6382 (TDD). USDA is an equal opportunity provider and employer.

* Corresponding author. Tel.: +1 806 746 5353; fax: +1 806 746 5028.
E-mail address: jeff.carroll@ars.usda.gov (J.A. Carroll).
† Present address. USDA Agricultural Research Service, US Meat Animal Research Center, Clay Center, NE, USA 68933.
‡ Present address. USDA Agricultural Research Service, Grazinglands Research Laboratory, El Reno, OK, USA 73036.

0739-7240/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.domaniend.2013.08.002

whereas HS decreased post-LPS concentrations of TNF-α in RO heifers compared to RO heifers housed at TN ($P = 0.008$). A tendency ($P < 0.06$) was observed for a breed by T_a interaction for IL-6 concentrations such that RO heifers had greater post-LPS concentrations of IL-6 than ANG heifers when housed at HS ($P = 0.020$). A breed by T_a interaction was observed for interferon-γ (IFN-γ; $P < 0.01$) concentrations such that HS decreased post-LPS concentrations of IFN-γ in ANG heifers compared to ANG heifers housed at TN ($P < 0.001$), and HS increased post-LPS concentrations of IFN-γ in RO heifers compared to RO heifers housed at TN ($P = 0.017$). These data indicate differences in the acute phase response between the heat-tolerant RO and heat-sensitive ANG heifers under different T_a which may aid in elucidating differences in productivity, disease resistance, and longevity among cattle breeds.

Published by Elsevier Inc.

1. Introduction

Strides have been made to increase knowledge on the immune response in livestock, particularly in cattle. As we gain a better understanding of the immune system, researchers and producers will be able to work together to create and (or) modify management practices to maximize cattle health and well-being while minimizing the negative effects of illness on productivity (average daily gain, dry mater intake, medical costs, etc). Several factors have been reported to modulate the immune response in cattle, including breed [1–3].

The use of heat-tolerant *Bos taurus* cattle breeds in breeding programs in subtropical regions of the United States is of increasing interest because they offer some favorable production traits compared with heat-tolerant *Bos indicus* breeds (ie, Brahman) [4–7]. Because differences exist between the immune response of *Bos taurus* and *Bos indicus* breeds, it is suspected that differences exist between diverse *Bos taurus* breeds as well [1]. A previous study reported that differences exist in the acute phase response of 2 diverse *Bos taurus* cattle breeds to a lipopolysaccharide (LPS) challenge which differ in heat tolerance [Angus (ANG; heat-sensitive) and Romosinuano (RO; heat-tolerant)] [3] when housed in a thermal neutral environment. Specifically, RO steers produced a greater rectal temperature (RT) response than ANG steers, whereas ANG steers produced a greater cortisol response to LPS challenge. In addition, the tumor necrosis factor-α (TNF-α) response was delayed and extended in RO steers compared to ANG steers. These results suggested that differences existed for the acute phase response of these 2 diverse *Bos taurus* breeds that differ in heat-tolerance, giving insight about other physiological, immunologic, and endocrine differences observed among cattle breeds.

As a follow-up on a previous study, the objective of the present experiment was to identify differences in the acute-phase response of the heat-tolerant RO and the heat-sensitive ANG heifers to a LPS challenge when housed at different air temperatures (T_a). The use of LPS, a component of the cell wall of gram-negative bacteria such as *Escherichia coli*, has been used in many research models to elicit an acute inflammatory response. The RO is a breed native to Colombia, South America, and derived its name from its origin in the Sinú river region (sinuano) of northern Colombia and its polled (romo) character [8]. The RO breed is noted for its longevity, docile temperament, and adaptation to tropical stressors [5,9,10]. Because of the differences in the acute phase response to LPS challenge observed previously between RO and ANG cattle when housed at thermal neutral conditions, it was hypothesized that during heat stress (HS), the RO heifers would be more heat tolerant than the ANG heifers, resulting in differences in the acute phase response between the 2 breeds.

2. Materials and methods

2.1. Experimental design

All experimental procedures were in compliance with the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* and approved by the Institutional Animal Care and Use Committee at the University of Missouri.

Eighteen-month-old ANG ($n = 11$; 306.7 ± 25.9 kg BW) and RO ($n = 10$; 312.9 ± 32.0 kg BW) heifers were transported from the USDA Agricultural Research Service SubTropical Agricultural Research Station (STARS) facility in Brooksville, Florida, to the Brody Environmental Chambers at the University of Missouri, Columbia. The ANG heifers used in the present study were from the USDA Agricultural Research Service STARS herd that descended from a group of cattle from the University of Florida obtained in the early 1950s. Beginning in 1955, ANG bulls from the Wye Plantation (Wye Mills, MD, USA) were used to generate (naturally) the STARS ANG cow herd. In the early 1970s and in the early 1990s, semen from modern Wye bulls was used on some of the cows to introduce different bloodlines. On the basis of this background information, the ANG cattle were purebred ANG cattle that were most likely not contaminated with genetics of other breeds over the years. Heifers were housed in separate stanchions in 4 temperature-controlled environmental chambers and allowed ad libitum access to feed and water throughout the study. Heifers were randomly placed in the environmental chambers within breed, with both breeds represented in each chamber. Air temperature was maintained within cycling thermoneutrality (TN; 18.5°C–23.5°C) for a 1-wk adjustment period (day 1–7), followed by an increase in 2 chambers to a cycling HS (24°C–38°C) for an additional 2 wk (day 8–21; [6]; Fig. 1). The temperature-humidity index for the HS chambers cycled from 70 to 78 and cycled from 64.5 to 67 for the TN chambers. Relative humidity was maintained below 50% in all chambers during the trial. Five ANG and 5 RO heifers were housed at TN, whereas 6 ANG and 5 RO heifers were housed at HS.

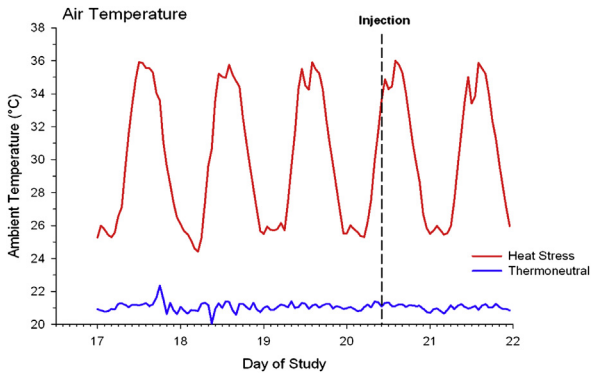

Fig. 1. Air temperatures in environmental chambers cycling at thermoneutrality (18.5°C–23.5°C) and heat stress (24°C–38°C). Administration of lipopolysaccharide (Injection) occurred at 10 AM on day 20 of the study.

On day 19, heifers were fitted with RT recording devices [11] that measured RT continuously at 1-min intervals and with jugular vein catheters and were then returned to their respective chambers. On day 20, heifers were administered 0.5 μg/kg BW LPS (from *E coli* O111:B4; Sigma-Aldrich, St Louis, MO, USA) intravenously at time 0 h (10 AM). Blood samples were collected in Sarstedt tubes with no additive (Sarstedt Inc, Newton, NC, USA) at 0.5-h intervals from −2 to 8 h and again at 24 h relative to LPS challenge at 0 h. Blood samples were allowed to clot for 30 min at room temperature and were then centrifuged at 1500 × *g* for 20 min at 4°C. Serum was isolated and stored at −80°C until analyzed for concentrations of cortisol, TNF-α, IL-6, and interferon-γ (IFN-γ). Sickness behavior scores (SBSs) were also determined by a single observer after the collection of each blood sample. Measurements of skin, ruminal, and vaginal temperature from this study were previously reported [12,13].

2.2. Sickness behavior scores

A trained observer assessed and recorded each heifer's SBS by visual observation after the collection of each blood sample. Heifers were scored on a scale of 1 (active or agitated), showing the least amount of sickness behavior, to 5 (lying on side with labored breathing), showing the greatest amount of sickness behavior (Table 1) [14]. Heifers were assigned SBSs by the same observer throughout the experiment.

Table 1
Sickness score definitions of visual signs of sickness.

Score	Description
1	Normal, alert, ears erect; head level or high, eyes open; standing, locomotor activity, responsive, performing maintenance behaviors
2	Calm but less alert, less activity, less responsive, standing or lying ventral, semilateral
3	Lying, calm, head distended or tucked, less alert, signs of some mild respiratory problems (coughing, wheezing)
4	Clinical signs of sickness, respiratory problems, not responsive, head distended, lethargic
5	All/most respiratory problems, mucus/foam; head distended, not responsive; medical intervention required

2.3. Serum analysis

Serum concentrations of cortisol were determined by enzyme immunoassay by using a commercially available kit validated for bovine samples according to the manufacturer's instructions (Arbor Assays, Ann Arbor, MI, USA). Serum concentrations of cortisol were determined by comparison to a standard curve of known concentrations. The minimum detectable concentration was 45.4 pg/mL, and intra-assay and interassay CVs were 0.7% and 1.8%, respectively. Concentrations are presented in nanograms per milliliter (ng/mL).

Serum cytokine concentrations (TNF-α, IFN-γ, and IL-6) were determined by a custom bovine-specific 3-plex sandwich-based chemiluminescence ELISA kit (Searchlight-Aushon BioSystems, Inc, Billerica, MA, USA). The minimum detectable concentrations were 0.5, 0.1, and 3.3 pg/mL for TNF-α, IFN-γ, and IL-6, respectively. All intra-assay CVs were <19%, and all interassay CVs were <22% for all assays. Concentrations are presented in picograms per milliliter (pg/mL).

2.4. Statistical analysis

Before analysis, RT data were averaged into 10-min intervals. Data were analyzed in 2 different periods: baseline (before LPS; time −2 to 0 h) and challenge (after LPS; 0 to 24 h). Data were analyzed by the MIXED procedure of SAS specific for repeated measures (SAS Institute Inc, Cary, NC, USA). Breed, T_a, time, and their interactions were included as fixed effects, with heifers within T_a (TN or HS) included as the subject. Specific comparisons were made between breed, T_a, time, and their interactions when significant with the use of the PDIFF option in SAS with the Tukey multiple comparison adjustment. A P value < 0.05 was considered significant and $0.05 < P < 0.10$ was considered a tendency. All data are presented as the least squares mean ± SEM.

3. Results

3.1. Rectal temperature

Before the administration of LPS, there was no effect of T_a ($P = 0.728$) or time ($P = 0.994$) on RT, but there was an effect of breed ($P < 0.001$) and a breed by T_a interaction ($P = 0.005$; Fig. 2). Specifically for the breed effect, ANG heifers had greater average RT (38.94°C ± 0.02°C) than RO heifers (38.64°C ± 0.02°C). For the breed by T_a interaction, HS resulted in a decrease in RT in RO heifers (38.60°C ± 0.03°C) compared to RO heifers housed at TN (38.68°C ± 0.03°C; $P = 0.034$), whereas there was a tendency ($P = 0.061$) for HS to increase RT in ANG heifers (38.97°C ± 0.02°C) compared to ANG heifers housed at TN (38.91°C ± 0.03°C).

After LPS administration, we observed an effect of T_a ($P < 0.001$), breed ($P < 0.001$), and time ($P < 0.001$) on RT. Specifically, RT increased in all heifers (time: $P < 0.001$) and was greater in ANG heifers (39.33°C ± 0.01°C) than in RO

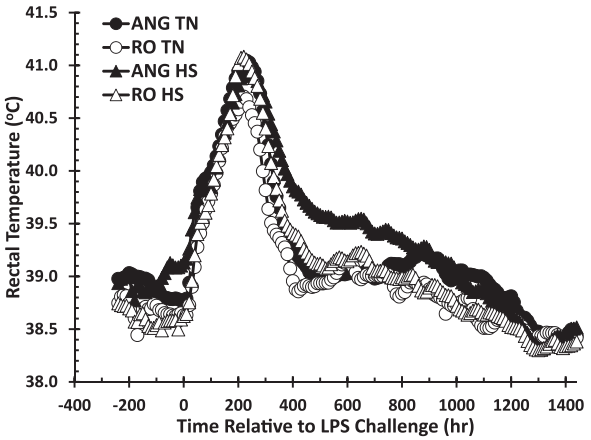

Fig. 2. Rectal temperature at 10-min intervals in ANG and RO heifers before and after an intravenous bolus injection of LPS (0.5 μg/kg BW) administered immediately after collection of a blood sample at time 0 hr. Heifers were housed at TN [18.5°C–23.5°C; ANG (n = 5) and RO (n = 5)] or cycling HS [24°C–38°C; ANG (n = 6) and RO (n = 5)]. For clarity of presentation, data are presented as least squares mean only. Before LPS challenge SEM = 0.03 and after LPS challenge SEM = 0.01. ANG, Angus; HS, heat stress; LPS, lipopolysaccharide; RO, Romosinuano; TN, thermoneutrality.

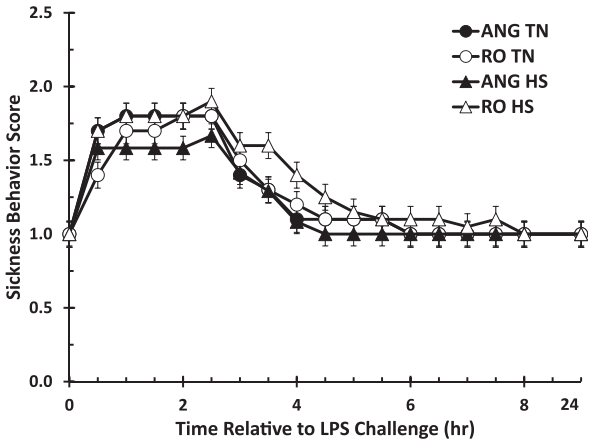

Fig. 3. Sickness behavior scores in ANG and RO heifers after an intravenous bolus injection of LPS (0.5 μg/kg BW) administered immediately after collection of a blood sample at time 0 hr. Heifers were housed at TN [18.5°C–23.5°C; ANG (n = 5) and RO (n = 5)] or cycling HS [24°C–38°C; ANG (n = 6) and RO (n = 5)]. ANG, Angus; HS, heat stress; LPS, lipopolysaccharide; RO, Romosinuano; TN, thermoneutrality.

heifers (39.11°C ± 0.01°C). In addition, RT was greater in heifers housed at HS (39.29°C ± 0.01°C) than heifers housed at TN (39.15°C ± 0.01°C). This resulted in a breed by T_a interaction ($P < 0.001$) such that the average RT after LPS challenge in RO heifers housed at TN was lower (39.05°C ± 0.01°C) than the RT of all other treatment groups ($P < 0.001$), whereas ANG heifers housed at HS had greater (39.41°C ± 0.01°C) average RT after LPS challenge than did all other treatment groups ($P < 0.001$).

3.2. Sickness behavior

Before administration of LPS, no differences were observed in SBSs among the heifers ($P = 1.000$ for T_a, breed, and time; all heifers had a score of 1 from −2 to 0 h; Fig. 3). After LPS challenge, SBS was affected by breed ($P = 0.001$) and time ($P < 0.001$) but not T_a ($P = 0.445$). In addition, a breed by T_a interaction ($P < 0.001$) was observed. Specifically, HS increased SBS after LPS challenge in RO heifers (1.36 ± 0.02) compared to RO heifers housed at TN (1.26 ± 0.02; $P < 0.001$), whereas HS decreased SBS after LPS challenge in ANG heifers (1.21 ± 0.02) compared to ANG heifers housed at TN (1.28 ± 0.02; $P = 0.014$).

3.3. Serum cortisol

Before administration of LPS, serum concentrations of cortisol were not affected by breed ($P = 0.204$) or time ($P = 0.411$), but they were affected by T_a ($P = 0.014$). Specifically, heifers housed at HS had greater cortisol concentrations (25.13 ± 2.23 ng/mL) than heifers housed at TN (16.63 ± 2.54 ng/mL; Fig. 4).

In response to LPS administration, cortisol concentrations were affected by T_a ($P = 0.009$), breed ($P = 0.032$), and time ($P < 0.001$). Specifically, cortisol concentrations increased in all heifers. Serum cortisol concentrations after LPS challenge were greater in heifers housed at TN (67.31 ± 2.04 ng/mL) than heifers housed at HS (60.09 ± 1.83 ng/mL) and were greater in RO heifers (66.63 ± 1.93 ng/mL) than in ANG heifers (60.76 ± 1.93 ng/mL). In addition, there was a tendency ($P = 0.084$) for a T_a by time interaction such that heifers housed at TN had greater cortisol concentrations than heifers housed at HS at 2 h ($P = 0.038$) and 3.5 h ($P < 0.001$) after administration of LPS.

3.4. Serum proinflammatory cytokines

Before administration of LPS, concentrations of pro-inflammatory cytokines were not affected by breed ($P = 0.428$–0.693), T_a ($P = 0.302$–0.507), or time ($P = 0.379$–

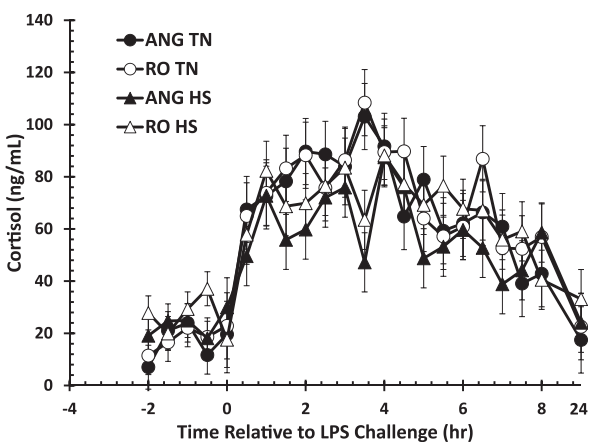

Fig. 4. Serum cortisol response in ANG and RO heifers after an intravenous bolus injection of LPS (0.5 μg/kg BW) administered immediately after collection of a blood sample at time 0 hr. Heifers were housed at TN [18.5°C–23.5°C; ANG (n = 5) and RO (n = 5)] or cycling HS [24°C–38°C; ANG (n = 6) and RO (n = 5)]. ANG, Angus; HS, heat stress; LPS, lipopolysaccharide; RO, Romosinuano; TN, thermoneutrality.

0.928; Fig. 5). After LPS challenge, concentrations of TNF-α were affected by time ($P < 0.001$) but were not affected by breed ($P = 0.622$) or T_a ($P = 0.460$; Fig. 5A). However, there was a breed by T_a interaction ($P < 0.001$). Specifically, HS increased post-LPS serum concentrations of TNF-α in ANG heifers (522 ± 52 pg/mL) compared with ANG heifers housed at TN (372 ± 52 pg/mL; $P = 0.041$), whereas HS decreased concentrations of TNF-α after LPS challenge in RO heifers (301 ± 58 pg/mL) compared to RO heifers housed at TN (537 ± 67 pg/mL; $P = 0.008$).

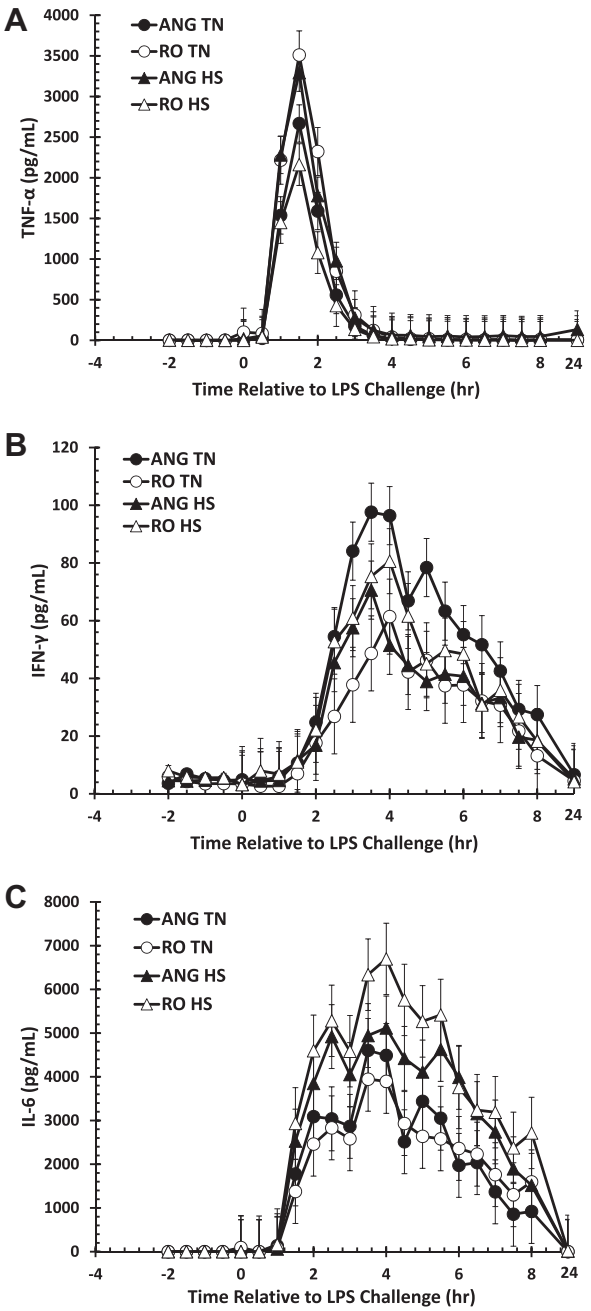

Fig. 5. Serum cytokine responses in ANG and RO heifers after an intravenous bolus injection of LPS (0.5 μg/kg BW) administered immediately after collection of a blood sample at time 0 hr. Heifers were housed at TN [18.5°C–23.5°C; ANG (n = 5) and RO (n = 5)] or cycling HS [24°C–38°C; ANG (n = 6) and RO (n = 5)]. Shown are serum cytokine response of (A) TNF-α, (B) IFN-γ, and (C) IL-6. ANG, Angus; HS, heat stress; IFN-γ, interferon-γ; LPS, lipopolysaccharide; RO, Romosinuano; TN, thermoneutrality; TNF-α, tumor necrosis factor-α.

After administration of LPS, serum IFN-γ concentrations were not affected by T_a ($P = 0.281$) but were affected by time ($P < 0.001$) and breed ($P = 0.012$; Fig. 5B). Specifically, ANG heifers produced greater serum IFN-γ concentrations (37.4 ± 1.6 pg/mL) than RO heifers (31.0 ± 1.9 pg/mL). In addition, a breed by T_a interaction ($P < 0.001$) was observed such that HS decreased concentrations of IFN-γ after LPS challenge in ANG heifers (30.0 ± 2.3 pg/mL) compared to ANG heifers housed at TN (44.7 ± 2.3 pg/mL; $P < 0.001$), and HS increased concentrations of IFN-γ after LPS challenge in RO heifers (35.6 ± 2.5 pg/mL) compared to RO heifers housed at TN (26.3 ± 2.9 pg/mL; $P = 0.017$).

Concentrations of IL-6 were not affected by breed ($P = 0.147$) but were affected by time ($P < 0.001$) and T_a ($P < 0.001$; Fig. 5C) after administration of LPS. Specifically, heifers housed at HS produced greater serum IL-6 concentrations (3175.8 ± 124.4 pg/mL) than heifers housed at TN (1967.8 ± 116.8 pg/mL). In addition, there was a tendency ($P = 0.053$) for a breed by T_a interaction such that RO heifers had greater concentrations of IL-6 (3466 ± 186 pg/mL) after LPS challenge than ANG heifers (2886 ± 165 pg/mL) when housed at HS ($P = 0.020$).

4. Discussion

An increase in body temperature is one of the most common signs observed in animals in response to an invading pathogen. This response is beneficial in that it helps to clear invading pathogens, such as bacteria, that are unable to thrive when the body temperature increases and may enhance certain immune functions that promote the removal of pathogens [15]. Although the main effect of T_a on RT was not significant before administration of LPS, HS reduced RT in RO heifers and tended to increase RT in ANG heifers. This suggests that the RO heifers housed at HS were more apt at maintaining a lower RT in the HS environment, whereas the RT of ANG heifers was unaffected by T_a before the LPS challenge. The breed effect observed before LPS challenge is similar to that observed by Scharf et al [6] when ANG and RO heifers were housed in the same chambers as in the present study and support the results that the RO breed is superior in its ability to regulate body temperature during both TN and HS environments. Scharf et al [6] suggested that the similarities between RO and *Bos indicus* cattle, such as Brahman, allowed for the RO heifers to dissipate heat through the skin because of shorter hair coats and greater blood flow to the skin. A greater heat loss to the environment would result in decreased RT in RO heifers as observed in the present study and supports the previous findings by Scharf et al [6]. The breed effect in RT observed before LPS administration in the present study is different than what was observed in ANG and RO steers housed at TN [3], which may be due to steers being used in the former study, and heifers being used in the present study, because breed differences in RT have previously

been published [14]. The breed effect in the present study was also significant after LPS challenge, with RO heifers maintaining lower RT than ANG heifers.

The temporal pattern of the RT response to LPS challenge is similar to what was observed in other studies in which LPS was administered to cattle [16–18]. The decreased RT in RO heifers housed at TN is in contrast to the response that was observed by Carroll et al [3] between RO and ANG steers housed at TN, in which RO steers produced a greater peak RT than ANG steers. It is possible that the difference observed between the present and the aforementioned studies is due to sex differences, because sex has been found to influence the RT responses to LPS challenge [19]. Specifically, it has been suggested that the programming of sex differences may be a result of differences in the pathways in the central nervous system that regulate fever and body temperature [20], perhaps related to differences in the manner in which heat is dissipated between males and females [21], or perhaps because of lean body mass composition differences between males and females [22]. In support of the results from the present study, Hammond et al [23] also reported a decreased RT in RO heifers compared with ANG heifers in a study conducted in the months of August and December at temperatures comparable with T_a in the environmental chambers used in the present study (TN and HS). The ability for RO heifers to produce a decreased RT response compared with ANG heifers, regardless of T_a, suggests that the heat-tolerant nature of the RO breed may have influenced the RT response after LPS challenge by allowing for greater heat dissipation from the body compared with ANG heifers. The ability to dissipate heat more efficiently may be beneficial because it may allow RO heifers to return to homeostasis at a faster rate, allowing for a quicker recovery from an infection.

Cattle often respond to infection through changes in behavior in response to increasing concentrations of cytokines. Concentrations of cytokines such as TNF-α and IL-6 are typically responsible for the decrease in neurovegitative functions such as eating and drinking that are often observed in response to infection [24]. This change in behavior allows animals to conserve energy for body processes that are more important for survival, such as the immune response that requires a significant amount of energy [25,26]. The difference in sickness behavior responses between heifers housed at HS compared with heifers housed at TN may be partially explained by cytokine concentrations, because IL-6 concentrations were greater in heifers housed at HS and also greater in RO heifers than in ANG heifers housed at HS. However, the TNF-α response in RO heifers housed at HS was decreased compared with the response of RO heifers housed at TN. Therefore, other factors, such as differences in energy reserves, may play a role in the sickness behavior responses observed in the present study. This is supported by data from a companion study which found that HS decreased glucose and NEFA concentrations in ANG heifers, yet increased NEFA concentrations in RO heifers when housed at HS [27].

The activation of the stress axis is necessary in response to an invading pathogen and subsequent inflammatory response to prevent a hyperinflammatory state, a condition that can be detrimental to the overall immune response. Therefore, the cortisol response to LPS challenge is important because it may influence the overall cytokine response. The greater cortisol concentrations in heifers housed at HS was expected, because T_a within the range used in the environmental chambers at HS conditions in the present study was previously found to cause a stress response [6,23,28]. The decreased cortisol response observed in heifers housed at HS compared with TN after administration of LPS likely indicates a blunted stress response due to the greater cortisol concentrations observed before LPS administration. Therefore, the greater cortisol concentrations observed before LPS administration in heifers housed at HS resulted in negative feedback on the hypothalamic-pituitary-adrenal axis, reducing the cortisol response stimulated by LPS administration. Greater cortisol concentrations observed in RO than in ANG heifers after LPS challenge in the present study is consistent with those reported by Hammond et al [23] in RO and ANG heifers during the months of August and December (representative of HS and TN temperatures in the present study). Hammond et al [23] suggested a relationship between cortisol concentrations and RT. To support this, acute stress (ie, due to handling) was found to increase RT in weaned calves [29]. As discussed previously, the breed effect on cortisol concentrations between RO and ANG heifers in the present study was complimentary to that observed by Hammond et al [23]; however, additional research is necessary to more fully understand the relationship between cortisol and RT as well as the influence of breed on this relationship.

The production of cytokines by cells of the innate immune system (ie, neutrophils and macrophages) is typically one of the first responses to be observed after infection, although detectable concentrations of cytokines are not usually evident for at least 30 min after LPS exposure. Therefore, the lack of significant effects on cytokine concentrations before administration of LPS is not surprising, because concentrations of these cytokines are typically negligible in the absence of a stimulus. The exposure to HS before and during the LPS challenge significantly affected cytokine responses after LPS challenge. The data from this study indicate that both T_a and breed can significantly affect the cytokine response to LPS challenge. The effect of breed on the TNF-α response and the lack of a breed effect on the IL-6 response of ANG and RO heifers housed at TN is similar to that observed in ANG and RO steers housed at TN [3]. However, the temporal pattern of cytokine secretion differed, likely because of the greater dose used by the aforementioned study (2.5 μg/kg BW compared with 0.5 μg/kg BW in the present study). Concentrations of TNF-α play a role in vasoconstriction; therefore, decreases in TNF-α in RO heifers in response to HS support the decreased RT observed in RO heifers before and after LPS challenge. However, TNF-α is known to stimulate production of other cytokines, such as IFN-γ and IL-6, which increased in RO heifers in response to HS. In addition, cortisol is well known to be anti-inflammatory, therefore reducing cytokine concentrations. It is possible that the greater concentrations of cortisol before the LPS challenge in heifers housed at HS modulated the cytokine response after LPS challenge. The decreased cortisol concentrations after LPS challenge in heifers housed at HS may have allowed for greater IL-6 concentrations in these

heifers after LPS challenge. However, cortisol concentrations do not explain the differences observed in TNF-α and IFN-γ. Both IL-6 and IFN-γ can stimulate the adaptive immune system through activation of T and B cells. Studies in mice and dairy cows have observed greater IL-6 concentrations when animals were exposed to HS conditions [30,31]. It is unclear what induces this increased secretion of IL-6 in response to HS, but it may possible play an immunoprotective role. The greater concentrations of IL-6 and IFN-γ and lesser concentrations of TNF-α in RO heifers exposed to HS suggest that RO heifers may have experienced reduced inflammation and greater activation of adaptive immunity in response to LPS challenge compared with ANG heifers.

5. Conclusions

When housed in an environment similar to HS conditions, RO heifers displayed greater SBSs and serum cortisol and IL-6 responses yet decreased febrile and TNF-α responses compared with ANG heifers. Overall, HS increased the febrile and serum IL-6 responses and decreased the serum cortisol response compared with heifers housed at TN. These data indicate differences in the acute phase response between heat-tolerant RO and heat-sensitive ANG breeds under different T_a, which may aid in elucidating differences in productivity, disease resistance, and longevity among cattle breeds.

Acknowledgments

The authors thank J.W. Dailey, J.C. Carroll, and C. Wu (USDA Agricultural Research Service) for technical assistance.

References

[1] Blecha F, Boyles SL, Riley JG. Shipping suppresses lymphocyte blastogenic responses in Angus and Brahman x Angus feeder calves. J Anim Sci 1984;59:576–83.
[2] Engle TW, Spears JW, Brown Jr TT, Lloyd KE. Effect of breed (Angus vs Simmental) on immune function and response to disease challenge in stressed steers and preweaned calves. J Anim Sci 1999;77:516–21.
[3] Carroll JA, Burdick NC, Reuter RR, Chase Jr CC, Spiers DE, Arthington JD, Coleman SW. Differential acute phase immune responses by Angus and Romosinuano steers following an endotoxin challenge. Domest Anim Endocrinol 2011;41:163–73.
[4] Spiers DE, Vogt DW, Johnson HD, Garner GB, Murphy CN. Heat-stress responses of temperate and tropical breeds of Bos taurus cattle. Arch Latinoam Prod Anim 1994;2:41–52.
[5] Chase Jr CC, Hammond AC, Olson TA, Murphy CN, Tewolde AJ, Griffin L. Introduction of Romosinuano in the U.S.A. Arch Latinoam Prod Anim 1997;5:54–71.
[6] Scharf B, Carroll JA, Riley DG, Chase Jr CC, Coleman SW, Keisler DH, Weaber RL, Spiers DE. Evaluation of physiological and blood serum differences in heat-tolerant (Romosinuano) and heat-susceptible (Angus) Bos taurus cattle during control heat challenge. J Anim Sci 2010;88:2321–36.
[7] Riley DG, Chase Jr CC, Coleman SW, Phillips WA, Miller MF, Brooks JC, Johnson DD, Olson TA. Genetic effects on carcass quantity, quality, and palatability traits in straightbred and crossbred Romosinuano steers. J Anim Sci 2012;90:2159–66.
[8] Rouse JE. The Criollo: Spanish cattle in the Americas. Norman, OK: University of Oklahoma Press; 1977.
[9] Elzo MA, Manrique C, Ossa G, Acosta O. Additive and nonadditive genetic variability for growth traits in the Turipaná Romosinuano-Zebu multibreed herd. J Anim Sci 1998;76:1539–49.
[10] Vogt DC, Murphy C, Crawford R, Clarke A, Cole E, Garner G, Tewolde A. Comparision of growth, carcass and meat characteristics of straightbred Angus and Romo Sinuano x Angus cross cattle managed under a forage system. Mt. Vernon, MO: University of Missouri Southwest Center Agricultural Experiment Station: Missouri Agricultural Experiment Station Southwest Center Research Report; 1991.
[11] Reuter RR, Carroll JA, Hulbert LE, Dailey JW, Galyean ML. Technical note: development of a self-contained, indwelling rectal temperature probe for cattle research. J Anim Sci 2010;88:3291–5.
[12] Chaffin RE, Scharf B, Johnson JS, Bryant JK, Kishore DK, Eichen PA, Carroll JA, Chase CC, Coleman SW, Burdick NC, Weaber RL, Spiers DE. A comparison of lipopolysaccharide-induced febrile response across heat-tolerant and -sensitive Bos taurus cattle in different thermal environments [Abstract M12]. J Anim Sci 2011;89(E-Suppl 1):7–8.
[13] Chaffin RE, Hoernig KH, Johnson JS, Bryant JK, Scharf B, Kishore DK, Eichen PA, Dierenfeld ES, Spiers DE. Use of infrared thermography to identify thermoregulatory differences between heat-sensitive and heat-tolerant breeds of Bos taurus cattle. J Anim Sci 2011;89(E-Suppl 1):465–6.
[14] Burdick NC, Carroll JA, Hulbert LE, Dailey JW, Ballou MA, Randel RD, Willard ST, Vann RC, Welsh Jr TH. Temperament influences endotoxin-induced changes in rectal temperature, sickness behavior, and plasma epinephrine concentrations in bulls. Innate Immun 2011;17:355–64.
[15] Hasday JD, Fairchild KD, Shanholtz C. The role of fever in the infected host. Microbes Infect 2000;2:1891–904.
[16] Carroll JA, Collier CT, Hulbert LE, Corley JR, Estefan AG, Fink DN, Johnson BJ. Yeast supplementation alters the health status of receiving cattle [Abstract T24]. J Anim Sci 2010;88(E-Suppl 2):315.
[17] Burdick NC, Carroll JA, Dailey JW, Randel RD, Falkenberg SM, Schmidt TB. Development of a self-contained indwelling vaginal temperature probe for use in cattle research. J Thermal Biol 2011;37:339–43.
[18] Burdick NC, Bernhard BC, Carroll JA, Rathmann RJ, Johnson BJ. Enhancement of the acute phase response to lipopolysaccharide challenge in steers supplemented with chromium. Innate Immun 2012;78:592–601.
[19] Carroll JA, Hulbert LE, Burdick NC, Caldwell LC, Dailey JW, Ballou MA, Vann RC, Welsh Jr TH, Randel RD. Bovine exhibit a sexually dimorphic innate immune response following an endotoxin challenge [Abstract 64]. J Anim Sci 2009;87(E-Suppl 2):21–2.
[20] Murakami N, Ono T. Sex-related differences in fever development in rats. Am J Physiol 1987;252:R284–9.
[21] Inoue Y, Tanaka Y, Omori K, Kuwahara T, Ogura Y, Ueda H. Sex- and menstrual cycle-related differences in sweating and cutaneous blood flow in response to passive heat exposure. Eur J Appl Physiol 2005;94:323–32.
[22] Lemire BB, Gagnon D, Jay O, Kenny GP. Differences between sexes in rectal cooling rates after exercise-induced hyperthermia. Med Sci Sports Exerc 2009;41:1633–9.
[23] Hammond AC, Olson TA, Chase Jr CC, Bowers EJ, Randel RD, Murphy CN, Vogt DW, Tewolde A. Heat tolerance in two tropically adapted Bos taurus breeds, Senepol and Romosinuano, compared with Brahman, Angus, and Hereford cattle in Florida. J Anim Sci 1996;74:295–303.
[24] Dantzer R. Cytokine-induced sickness behavior: where do we stand? Brain Behav Immun 2001;15:7–24.
[25] Borderas TF, de Passillé AM, Rushen J. Behavior of dairy cows after low doses of bacterial endotoxin. J Anim Sci 2008;86:2920–7.
[26] Carroll JA, Forsberg NE. Influence of stress and nutrition on cattle immunity. Vet Clin Food Anim 2007;23:105–49.
[27] Burdick Sanchez NC, Chaffin R, Carroll JA, Chase Jr CC, Coleman SW, Spiers DE. Heat-tolerant versus heat-sensitive Bos taurus cattle: Influence of air temperature and breed on the metabolic response to a provacative immune challenge. http://dx.doi.org/10.1016/j.domaniend.2013.08.005.
[28] Alvarez MB, Johnson HD. Environmental heat exposure on cattle plasma catecholamine and glucocorticoids. J Dairy Sci 1973;56:189–93.
[29] Crookshank HR, Elissalde MH, White RG, Clanton DC, Smalley HE. Effect of transportation and handling of calves upon blood serum composition. J Anim Sci 1979;48:430–5.
[30] Welc SS, Phillips NA, Oca-Cossio J, Wallet SM, Chen DL, Clanton TL. Hyperthermia increases interleukin-6 in mouse skeletal muscle. Am J Phys Cell Phys 2012;15:C455–66.
[31] Caroprese M, Albenzio M, Marino R, Santillo A, Sevi A. Dietary glutamine enhances immune responses of dairy cows under high ambient temperature. J Dairy Sci 2013;96:3002–11.